

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau



17

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁷ : C12Q 1/68	A1	(11) International Publication Number: WO 00/39346 (43) International Publication Date: 6 July 2000 (06.07.00)
(21) International Application Number: PCT/US99/31276 (22) International Filing Date: 30 December 1999 (30.12.99) (30) Priority Data: 60/114,399 31 December 1998 (31.12.98) US (71) Applicant: ICONIX PHARMACEUTICALS, INC. [US/US]; 850 Maude Avenue, Mountain View, CA 94043 (US). (72) Inventors: MELESE, Teri; Iconix Pharmaceuticals, Inc., 850 Maude Avenue, Mountain View, CA 94043 (US). PERKINS, Edward, L.; Iconix Pharmaceuticals, Inc., 850 Maude Avenue, Mountain View, CA 94043 (US). TUGENDREICH, Stuart; Iconix Pharmaceuticals, Inc., 850 Maude Avenue, Mountain View, CA 94043 (US). (74) Agents: ROBINS, Roberta, L.; Robins & Associates, Suite 200, 90 Middlefield Road, Menlo Park, CA 94025 (US) et al.		(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
(54) Title: METHOD FOR GENERATING A PATHWAY REPORTER SYSTEM		
(57) Abstract		
Methods and nucleic acid constructs for testing and validating activity reporter cells, using a plurality of host cell genes, are described.		

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

METHOD FOR GENERATING A PATHWAY REPORTER SYSTEM

5

Field of the invention

This invention relates to the fields of microbiology and drug discovery. More particularly, the invention relates to methods for preparing assay vehicles for investigating
10 gene function.

Background of the Invention

It is often difficult to determine the function of a gene in an organism, as many genes interact in complex webs with overlapping pathways. One can study genes by isolating nucleic acids and transferring them to a foreign host cell, which is less likely to respond to the transferred gene, but may still exhibit some response. However, some genes fail to exhibit any detectable change in the host cell, for example due to alternate metabolic or signaling pathways available to the host cell.
15

Screening for therapeutically useful compounds has commonly used biochemical screening and/or whole cell screening, in which cells are contacted with a compound under conditions which are believed to be relevant to the intended use of the compound and the cells are monitored for a particular readout which is indicative of an active compound.
20 However, it is often difficult to design an assay that provides a useful readout. For example, one can arrange an assay for an isolated surface receptor that determines when a test compound binds to the target receptor, but simple binding does not indicate that the
25 receptor is also activated or inhibited by the test compound.

Summary of the Invention

We have now invented a method for modifying host cells having a transfected gene so that a detectable phenotype is produced.

One aspect of the invention is a method for preparing a plurality of assays, by
5 transforming a plurality of host cells with nucleic acid constructs comprising a host cell gene linked to a detectable reporter (and optionally to a selectable marker and/or an affinity label) to provide a plurality of reporter cells, and transforming the reporter cells with a heterologous gene to provide a plurality of different transformed reporter cells. The transformed reporter cells are then selected for modulation of the detectable label
10 expression (or affinity label expression, or selection due to the selectable marker) as a result of the heterologous gene activity. Preferably, the transformed reporter cells are selected based on modulation that differs under different selected culture conditions.

Another aspect of the invention is a method for examining the activity of a heterologous gene in a host cell, by transforming a plurality of host cells containing said heterologous gene with a plurality of nucleic acid constructs, each said construct comprising a
15 different host gene operatively linked to a detectable label, and optionally to a selectable marker and an affinity label. The resulting transformants are subjected to variations in culture conditions (for example, changes in temperature, nutrients, crowding, chemicals, proteins, and the like), and transformants that exhibit a change in label expression as a
20 function of culture conditions are selected. The method enables one to determine all host genes that interact with the heterologous gene (or its product).

Another aspect of the invention is a nucleic acid construct useful in the method of the invention, comprising a host cell gene, a detectable label, and optionally a selectable marker and affinity label. The construct is preferably flanked by recombinase recognition
25 sites, and preferably further comprises appropriate maintenance and replication sequences sufficient for propagation in cloning and expression hosts.

Another aspect of the invention is a method for determining the biological effect of a compound, by contacting a panel of host cells with the compound, and determining the

change (if any) in expression of a detectable label, wherein each host cell comprises a heterologous gene and a detectable label, wherein the label is expressed in response to activation of a host cell gene by the heterologous gene (or its product).

Another aspect of the invention is a method for predicting the activity of a heterologous gene, by providing a panel of reporter cells as described above, transforming the reporter cells with a plurality of different heterologous genes of known function, and determining which heterologous genes are associated with activity in the reporter cells. The unknown gene is also transformed into a plurality of reporter cells, and its function determined by similarity to a gene of known function, where said similarity is based on the reporter cells activated by said genes.

Detailed Description

Definitions:

The term "essential gene" as used herein refers to a gene whose function is required for viability of its host, i.e., the host cell dies if the essential gene function is lost.

The term "detectable label" as used herein generally refers to a gene that encodes a product which can be detected by optical or fluorescent techniques, or by performing simple enzymatic assays (for example, lacZ). Detectable labels preferably exhibit characteristic spectra that permits their use in FACS and/or other optical-based sorting systems.

The term "affinity marker" refers to a gene encoding a protein, polypeptide, or epitope having binding characteristics that permit one to sort the protein by means of an affinity column. Exemplary affinity markers include, without limitation, HA, avidin, biotin, streptavidin, and the like.

The term "selectable marker" as used herein refers to a gene encoding a protein essential to survival of the host cell (or alternatively, capable of killing the host cell under specific conditions). Suitable selectable markers include HIS3, thymidine kinase, and the like.

The terms "DNA array" and "microarray" are used interchangeably to refer to devices capable of detecting the presence of one or more nucleic acid sequences in a sample, such as, for example, the DNA chip technology commercialized by Affymetrix. "Array" as used herein refers to a plurality of objects arranged in a pattern, in which
5 different objects are distinguished by their position in the pattern. Arrays are often set out in two-dimensional grids, but may be arranged in any way desired.

The term "ARC" or "activity reporter cell" refers to a host cell containing a heterologous gene, in which the heterologous gene produces a detectable phenotype in the host cell. The phenotype varies in response to an additional factor, which can be environmental (for example, temperature, cell contact, and the like), chemical, or the presence of
10 additional heterologous genes in the host.

The term "recombinase" refers to an enzyme which cleaves nucleic acids at a specific recognition site or sequence, facilitating integration of a nucleic acid into a host cell genome. Exemplary recombinases include, without limitation, cre.
15

General Method:

The technology for using yeast as a surrogate host to express foreign proteins is now well established. However, there still exists a need for methods to assess the genome-wide impact of a protein on the host cell's physiology, particularly for proteins
20 of unknown function. The instant invention (PRIYSM) is designed to report the effect of heterologous gene expression on cellular pathways in the surrogate host, and represents an improvement over technologies based on DNA microarrays ("chips"). DNA chips tend to be static, and to provide a readout at only a single point in time (or at selected points), whereas the method of the invention is capable of providing a continuous read-
25 out. Information derived using the method of the invention can be used to design genetic tests to establish relationships between multiple heterologous genes and compounds.

The application of PRIYSM for reporting the genomic effects of heterologous gene expression in a surrogate host involves constructing a yeast genomic library in a

transposon tagging system (for example, an E. coli based transposon tagging system), transposon tagging the yeast genomic library, introducing the transposon-tagged gene fusion library constructs into yeast, screening for appropriate reporter-linked cellular readouts, and applying the PRIYSM technology to globally monitor the effects of
5 heterologous gene expression.

In the initial stage, a library is constructed consisting of target nucleic acids (for example, host genomic DNA fragments) of approximately 5 Kb in size cloned into a modified shuttle vector (e.g., an E. coli/yeast shuttle vector). The shuttle vector contains all the required factors necessary for plasmid maintenance in E. coli and some required for
10 the host, for example an E. coli replication origin and antibiotic resistance marker, as well as a yeast centromere and a yeast autonomous replication sequence. The eukaryotic host genomic fragments are cloned into the plasmid, and the library propagated in an E. coli host. The eukaryotic host genomic fragments are inserted flanked by loxP sites if cre recombinase is to be used, or other sites recognized by the recombinase enzyme to be
15 used if other than cre. The library is constructed such that there is a sufficient number of cloned transformants to guarantee a probability greater than 99% that complete coverage of the eukaryotic host genome will be included. Where the eukaryotic host is yeast, this is approximately 20,000 recombinants. The E. coli host is selected to provide all the genetic factors necessary for transposon tagging of the eukaryotic host genomic fragments, as
20 well as the necessary enzymes for catalyzing transposition and resolution (provided in trans). Examples of these types of yeast transposon tagging systems include the Tn10 based "lambda hopping system" and the Tn3 transposon tagging system (O. Huisman et al., Genetics (1987) 116(2):191-99; P. Ross-Macdonald et al., Proc Natl Acad Sci USA (1997) 94:190-95).

25 Ross-Macdonald et al. (supra) described a transposon tagging system employing Tn3, a green fluorescent protein (GFP) and a hemagglutinin antigen epitope tag (HA) adjacent to a yeast selectable marker. When this element transposes in-frame to a yeast gene, a recombinant fusion protein is generated consisting of the yeast gene product fused

to the GFP-HA element. The instant method in general employs a detectable label (such as, for example, GFP or a variant thereof), an affinity marker or antigen (such as, for example, HA), and further includes a selection marker, such as a yeast URA3 gene fused in-frame, such that a functional URA3 protein is produced only if inserted in-frame into a yeast gene.

The resulting transposon construct is then transposed into the host genome fragment library, following standard protocols. Successful transpositions introduce a yeast selectable marker into the plasmids. Following the subsequent purification of the host genomic library (containing the random insertion of transposable elements), the library is transformed into a matching eukaryotic host (e.g., yeast), utilizing the selectable marker inserted into the transposon element to generate potential eukaryotic gene fusion reporter-linked strains, where the gene fusions are propagated as autonomous replicating DNA molecules. Approximately 100,000 transformants are typically sufficient. The transformants are isolated and inoculated into microtiter dishes to serve as a first layer for arraying the possible reporter-linked strains. Utilizing microarray technology, the transformants can be "printed" onto soft agar growth media to form intermediate "chip" arrays. These intermediate arrays are then exposed to various stress conditions, whether by varying the environment, or by providing a varying environment as part of the "chip" (e.g., by establishing one or more chemical concentration gradients across the chip). Host cells that contain gene fusions that respond to the various conditions are identified as those that demonstrate an increase or decrease in fusion gene expression (determined, for example, by fluorescence microscopy utilizing the GFP construct). The identified host cells are then re-arrayed in order to generate a panel of gene fusion constructs that can globally monitor the effect of heterologous gene expression on cellular pathways in the surrogate host. Finally, the reporter gene fusions can be integrated into the host genome by transforming the cells with a second plasmid expressing the appropriate recombinase (e.g., cre recombinase). The recombinase facilitates integration of the gene fusion into the host genome.

The resulting panel is useful for examining activity reporter cells (ARCs), which contain one or more heterologous genes which produce a phenotype in the host cell (where the phenotype depends on the biological activity of the heterologous gene). See USSN 09/187918, filed 7 November 1998, incorporated herein by reference in full. The reporter panel can also be generated "manually" by isolating the promoters from some or all of the host's genes by PCR, and individually linking them to the reporter gene. Since the heterologous gene may affect a variety of host genes, the panel of the invention provides a means for assaying that activity. Once the PRIYSM panel is established, a surrogate host containing the heterologous gene can be easily mass mated to the panel of reporter linked constructs, or otherwise transformed with the reporter constructs. The resulting mated host cells can be arrayed again, for example into soft agar, and the heterologous gene expressed. Again, fluorescence microscopy can be used to identify reporter constructs whose expression is altered by the heterologous gene. This results in a genetic network of cell-based reporters for each heterologous gene tested. Alternatively, the panel itself can be transfected with a heterologous gene (or construct) directly, thus forming ARCs in situ. Such transformation can be performed on the panel as a pool of cells or arranged in an array.

Additionally, reporters can be selected directly in ARCs, including ARCs that fail to demonstrate an obvious phenotype. For this, the constructed gene fusion library is transformed directly into the host strain containing the heterologous gene. Upon expression of the heterologous gene, the affected reporters can easily be identified either by direct selection for or against URA3 function (including, for example, identification using a DNA array), or can be sorted using FACS or similar technologies, employing the GFP. The identified reporters can then be arrayed to generate a PRIYSM panel specific for each heterologous gene. This approach circumvents the requirement of a growth interference/complementation phenotype, and directly establishes multiple reporter linked assays for each heterologous gene. Finally, the identified reporters can be integrated into the host genome by the cre-lox method set forth above.

The method of the invention can also be applied to essential genes, by omitting any integration step. Integration into an essential gene can cause loss of function, with resulting death of the host cell. However, in the present method, the PRIYSM constructs can be used in plasmid form, without requiring integration into the host cell genome.

5 In contrast to current array technology, which provides only a readout at a given point in time, the method of the invention can provide continuous data, a physiological readout of a set of chosen cellular pathways, without relying on a growth readout. Further, PRIYSM is genetically tractable, and extends the use of global reporting. The three-part fusion constructs employed in the invention (e.g., GFP-URA3-HA) enables one to
10 use any fusion construct whose expression is modulated or altered by a heterologous gene as a functional tool, using selection based upon prototrophy (or by cell sorting using the marker) provides multiple entry points for ARC expansion (for example, cloning more members of a protein family which has been found to induce a particular reporter) using chemicals and/or other expressed genes. More importantly, this expansion can be directed
15 to any or all of the entry points, allowing a greater degree of precision for ARC expansion. For example, if the initial PRIYSM analysis of an ARC reveals that a subset of the panel is altered, each point of that subset can be genetically screened by either compounds or additional genes that affect that specific point in the subset. The screening of additional genes against the original ARC phenotype, or any point in the PRIYSM panel
20 subset, can establish genetic epistasis and identify novel members in the genetic pathways. In addition, compounds identified on the basis of ARC phenotype reversal can quickly be screened with the PRIYSM panel to determine if the compound directly counteracts the heterologous protein (such that all points in each ARC network are altered) or if the compound effects are indirect (affecting only a few points in the ARC
25 network). Finally, since PRIYSM technology does not rely on a growth readout, heterologous genes that do not yield an altered growth phenotype can still be analyzed based on their effect with the PRIYSM panel.

What is claimed:

- 1.) A method for determining genetic pathways in a host cell, comprising:
 - a) providing a plurality of first host cells;
 - 5 b) transforming said first host cells with a plurality of different nucleic acid constructs, wherein said constructs comprise a plurality of different host cell genes, each operatively linked to a polynucleotide encoding an detectable label;
 - c) culturing said transformed cells under altered conditions sufficient to alter expression of a gene in said host cell; and
 - 10 d) selecting cells which exhibit said label in response to said altered conditions.
- 2.) The method of claim 1.), wherein said host cells comprise eukaryotic cells.
- 3.) The method of claim 2.), wherein said eukaryotic host cells comprise yeast.
- 15 4.) The method of claim 1.), wherein said first host cells further comprise a heterologous gene.
- 5.) The method of claim 4.), wherein said heterologous gene is a human gene.
- 20 6.) The method of claim 1.), wherein said nucleic acid construct further comprises a selectable marker.
- 7.) The method of claim 6.), wherein said nucleic acid construct further comprises an
25 affinity label.

- 8.) The method of claim 7.), wherein said nucleic acid construct encodes HA, GFP, and URA3.
- 9.) The method of claim 1.), wherein said plurality of host cell genes comprises at least
5 50% of the genes found in said host.
- 10.) The method of claim 9.), wherein plurality of host cell genes comprises at least 80% of the genes found in said host.
- 10 11.) The method of claim 10.), wherein said plurality of host cell genes comprises substantially all of the genes found in said host.
- 12.) The method of claim 1.), wherein said nucleic acid constructs are integrated into the host cell genome.
- 15 13.) The method of claim 1.), further comprising:
integrating said nucleic acid constructs into the genomes of said host cells.
- 14.) The method of claim 1.), further comprising:
20 providing a second host cell, comprising a heterologous gene; and
mating said first host cells and said second host cells.
- 15.) The method of claim 14.), wherein said heterologous gene comprises a human gene.
- 25 16.) The method of claim 15.), wherein said heterologous gene comprises a plurality of human genes.

- 17.) The method of claim 1.), wherein said altered conditions are selected from the group consisting of altered osmolarity, altered culture temperature, radiation, presence of virus, and presence of a chemical.
- 5 18.) A nucleic acid construct for determining the effect of a heterologous gene on a selected host cell, comprising:
- a) A host cell gene;
 - b) A detectable label operatively linked to said host cell gene; and
 - c) A selectable marker gene.
- 10 19.) The nucleic acid construct of claim 18.), further comprising an affinity label.
- 20.) The nucleic acid construct of claim 18.), further comprising a recombinase recognition site flanking each end of said construct.
- 15 21.) The nucleic acid construct of claim 19.), further comprising a recombinase recognition site flanking each end of said construct.
- 20 22.) The nucleic acid construct of claim 21.), wherein said recognition site is a cre-lox site.

INTERNATIONAL SEARCH REPORT

Intern. Application No
PCT/US 99/31276

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 39483 A (VENTANA GENETICS INC) 11 September 1998 (1998-09-11) see whole doc. esp. claims and examples	1-22
X	US 5 569 588 A (ASHBY MATTHEW ET AL) 29 October 1996 (1996-10-29) the whole document	1-22
A	WO 97 13877 A (LYNX THERAPEUTICS INC ; MARTIN DAVID W (US)) 17 April 1997 (1997-04-17) the whole document	
A	WO 95 20681 A (INCYTE PHARMA INC) 3 August 1995 (1995-08-03) the whole document	
	-/-	

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *A* document member of the same patent family

Date of the actual completion of the international search

7 June 2000

Date of mailing of the international search report

14/06/2000

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Müller, F

INTERNATIONAL SEARCH REPORT

Intern. Appl. Application No

PCT/US 99/31276

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	KAIN S R ET AL: "GREEN FLUORESCENT PROTEIN AS A REPORTER OF GENE EXPRESSION AND PROTEIN LOCALIZATION" BIOTECHNIQUES,US,EATON PUBLISHING, NATICK, vol. 19, no. 4, 1 October 1995 (1995-10-01), pages 650-655, XP002033687 ISSN: 0736-6205 the whole document	
A	SCHENA M ET AL: "PARALLEL HUMAN GENOME ANALYSIS: MICROARRAY-BASED EXPRESSION MONITORING OF 1000 GENES" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA,US,NATIONAL ACADEMY OF SCIENCE. WASHINGTON, vol. 93, no. 20, 1 October 1996 (1996-10-01), pages 10614-10619, XP002912238 ISSN: 0027-8424 the whole document	
P,X	WO 99 31277 A (DOWER STEVEN ;QUARNSTROM EVA (GB); MEDICAL SCIENCE SYSTEMS INC (US) 24 June 1999 (1999-06-24) see whole doc. esp. claims	1-22

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 99/31276

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9839483 A	11-09-1998	US 5955275 A	21-09-1999
		AU 6543898 A	22-09-1998
		EP 0973943 A	26-01-2000
		NO 994290 A	03-11-1999
		US 6025485 A	15-02-2000
US 5569588 A	29-10-1996	AU 6720996 A	05-03-1997
		CA 2202154 A	20-02-1997
		EP 0791078 A	27-08-1997
		JP 10507647 T	28-07-1998
		WO 9706277 A	20-02-1997
WO 9713877 A	17-04-1997	AU 712929 B	18-11-1999
		AU 4277896 A	06-05-1996
		AU 6102096 A	30-12-1996
		AU 7717596 A	30-04-1997
		CN 1193357 A	16-09-1998
		CZ 9700866 A	17-09-1997
		CZ 9703926 A	17-06-1998
		EP 0793718 A	10-09-1997
		EP 0832287 A	01-04-1998
		EP 0931165 A	28-07-1999
		FI 971473 A	04-06-1997
		HU 9900910 A	28-07-1999
		JP 11507528 T	06-07-1999
		JP 10507357 T	21-07-1998
		NO 971644 A	02-06-1997
		NO 975744 A	05-02-1998
		PL 324000 A	27-04-1998
		WO 9641011 A	19-12-1996
		AU 718357 B	13-04-2000
WO 9520681 A	03-08-1995	US 5840484 A	24-11-1998
		AU 688465 B	12-03-1998
		AU 1694695 A	15-08-1995
		BG 100751 A	31-07-1997
		BR 9506657 A	16-09-1997
		CA 2182217 A	03-08-1995
		CN 1145098 A	12-03-1997
		CZ 9602189 A	14-05-1997
		EP 0748390 A	18-12-1996
		FI 962987 A	26-09-1996
		JP 9503921 T	22-04-1997
		LV 11696 B	20-08-1997
		NO 963151 A	27-09-1996
		PL 315687 A	25-11-1996
		HU 75550 A	28-05-1997
WO 9931277 A	24-06-1999	NONE	